REMARKS

Claims 1, 2, 4-22, and 25-35 are pending in this application. Claim 17-22 have been withdrawn from consideration by the Examiner.

Claims 1, 4, 30-31 and 34 have been amended. New claims 35-36 have been added. Support for the amendments to the claims may be found throughout the application, for example, in the specification at page 26, lines 4-7 and in Tables 1, 2 and 3.

No new matter has been added by the amendments.

I. The Rejection of Claims 1-2, 4-11, 16 and 28-29, Under 35 U.S.C. § 103(a) Should be Withdrawn

Claims 1-2, 4-11, 16 and 28-29 are rejected under 35 U.S.C. 103(a) as allegedly obvious over U.S. Patent No. 5,705,366 to Backus *et al.* ("Backus") in view of Bustin *et al., Journal of Molecular Endocrinology*, 2000, vol. 25, p. 169-193 ("Bustin") and further in view U.S. Patent No. 5,773,258 to Birch *et al.* ("Birch").

According to the Office Action, Backus discloses a method for the coamplification of two or more target nucleic acids having different sequence compositions. The Office Action concedes, however, that Bakus does not disclose the use of a chemically-modified thermostable DNA polymerase. The Office Action attempts to cure this deficiency with the disclosure of Birch, suggesting that Birch discloses the use of a Hot start DNA polymerase.

The Office Action further concedes that "neither Backus nor Birch explicitly teach that the two or more target nucleic acids are present in comparable copy numbers and the highest copy number is 10 fold." Office Action at page 7, fourth paragraph. However, the Examiner argues that Bustin discloses, "the coamplification of nucleic acids which are present at comparable copy numbers, wherein the maximum difference between the lowest and highest copy number is 10-fold . . . ." Office Action at page 8, second full paragraph.

The Examiner concludes that, "one of ordinary skill in the art at the time the invention was made would have been motivated to extend the coamplification in the presence of a polymeric volume exclusion agent taught by Backus to incorporate the teachings of Bustin with

a reasonable expectation for success in order to achieve quantitative coamplification of two nucleic acid targets of similar copy number." Office Action at page 9, first paragraph.

Applicants respectfully disagree and traverse this rejection for at least the following reasons.

Even if the Office were to have made a *prima facie* case of obviousness (which Applicants do not concede that it has), the presently claimed methods provide results that are unexpectedly superior to those that could have reasonably been expected. A *prima facie* showing of obviousness may be rebutted by demonstrating "that the claimed invention exhibits some superior property or advantage that a person of ordinary skill in the relevant art would have found surprising or unexpected." *In re Soni*, 54 F.3d 746, 750 (Fed. Cir. 1995).

As discussed in the 2002 multiplex PCR review article by Markoulatos *et al.* in *Journal of Clinical Laboratory Analysis 16*:47–51 (2002) ("Markoulatos"), the authors indicated that "common problems encountered in multiplex PCR [are] spurious amplification products, uneven or no amplification of some target sequences, and difficulties in reproducing some results . . . ." Markoulatos at Abstract. As such, the authors cautioned that "development of an efficient multiplex PCR usually requires strategic planning and multiple attempts to optimize reaction conditions." Markoulatos at Abstract. Markoulatos further indicates that:

> the relative concentration of the primers, concentration of the PCR buffer, balance between the magnesium chloride and deoxynucleotide concentrations, cycling temperatures, and amount of template DNA and Taq DNA polymerase are important. An optimal combination of annealing temperature and buffer concentration is *essential* in multiplex PCR to obtain highly specific amplification products.

Markoulatos at Abstract. Markoulatos also indicates that the "list of various factors that can influence the reaction is *by no means complete*." *Id.* (emphasis added). Indeed, Bustin similarly concludes that, "successful multiplexing is *never* trivial and *requires* careful consideration about the suitability of both chemistry and instrumentation." Bustin at 185, first column, second paragraph (emphasis added).

As discussed above, Bustin concludes that, "if quantification is the main aim, it is probably best to limit multiplexing to the detection of *two* or *three* transcripts." Bustin at 185,

right col. second paragraph, while Backus only discloses the amplification of four targets, due to the difficulties associated with optimizing multiplex PCR.

Applicants also appreciated these problems, noting that the presence of many different primers leads to a high probability of primer dimer formation. As stated in the present specification, "[t]he presence of primer dimers dramatically reduces the efficiency of the reaction." Specification at page 6, lines 22-25. Applicants further indicated that, "[e]fficient co-amplification of multiple targets (multiplex PCR) is only possible when reaction conditions are chosen that allow all reactions to take place simultaneously and all reactions only minimally influence each other." *Id.* at lines 27-29.

In light of the disclosures of the Backus, Bustin and Markoulatus, discussed herein, the skilled artisan would have believed that a fair amount of luck and an enormous amount of optimization over a *long and incomplete* list of variables, conditions and parameters would be required order to develop a new multiplex PCR assay. The skilled artisan would have been further discouraged by the thought of attempting to design a multiplex PCR reaction capable of amplifying two or more different target nucleic acids present at *comparable* copy numbers because such experiments usually require tedious primer molar ration determination experimentation.

Nevertheless, the Office Action continues to assert that "[a] *need* for optimization of a multiplex amplification is not unexpected . . . and does not provide any basis for a lack of reasonable expectation of success. One of ordinary skill would understand the factors that need to be adjusted to optimize any type of multiplex amplification." Office Action at page 21, first full paragraph (emphasis added). Applicants respectfully disagree with this assertion.

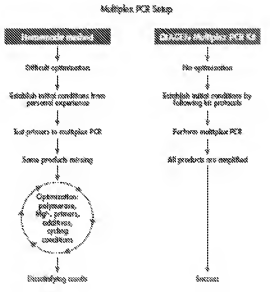
Applicants assert that it is precisely the *lack of a need* for optimization that renders the results obtained by the claimed methods unexpectedly superior to what would have been expected by one of skill in the art.

The claimed methods represent a rapid and efficient method that allows the skilled artisan to conduct a multiplex PCR assay in which two or more target sequences could be simultaneously amplified with *little or no* optimization work required. These unexpected and superior results are discussed in "New QIAGEN® Multiplex PCR Kit" *QIAGEN® News* 5:13-16 (November 2002) (hereinafter "QIAGEN News," Exhibit A):

> The new QIAGEN® Multiplex PCR Kit is the first kit specifically developed for multiplex PCR. The simple multiplex master-mix solution *eliminates* the need for lengthy optimization procedures, such as adjusting the amounts of Mg^{2+} and enzyme or even, as frequently required, adjusting primer concentrations. Now standard multiplex PCR applications are fast and easy to perform. (emphasis added)

QIAGEN News at page 13, first column, second paragraph.

The Applicants compared the claimed methods with the difficult prior art optimization procedures associated with multiplex PCR in a cartoon in Qiagen News.



As opposed to the "tedious" prior art methods which "require extensive optimization" often leading to "disappointing" results (*see* QIAGEN News, p. 14, left col.), the claimed methods "eliminate the need for length optimization procedures" and are "fast and easy to perform." (*See* QIAGEN News, p. 13, left col.). Indeed, the claimed methods require "no optimization" (see Qiagen News) and are "virtually eliminated." See "Highly Efficient Multiplex PCR Using Novel Reaction Chemistry," Agilent Technologies ©2003 at page. 4, left column. ("Engel," Exhibit B).

Engel states that the claimed methods were used to amplify "up to 19 targets in a single PCR step under standard conditions." *Id* at p. 1. On page 15 of QIAGEN News, the Applicants describe experimental results using standard kit reagents of the claimed methods wherein 16 nucleic acid targets ranging from 99-995 bp are simultaneously and successfully amplified. Both panels "A" and "B," show the results of a multiplex PCR reaction on 16 nucleic acid targets with

varying amounts of a hot-start polymerase obtained from supplier A_{II} as well as varying $MgCl_2$ concentrations.

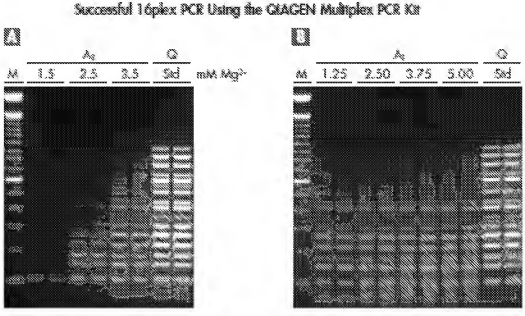


Figure 1 *Multiplex PCR of 16 targets (99–955 bp) was carried out for 35 cycles using standard conditions (5kU) for the QIAGEN Multiplex PCR Kit (Q) without further optimization or using a variety of conditions with a hotstart DNA polymerase from Supplier A_{II} (A).* A *Comparison using 2.5 units/50 µl reaction of the hotstart DNA polymerase from Supplier A_{II} and with the indicated Mg^{2+} concentrations.* B *Comparison using the optimized Mg^{2+} concentration (3.5 mM) for the hotstart DNA polymerase from Supplier A_{II} and the indicated amounts of enzyme per 50 µl reaction. M: marker.*

Only the reactions using the Applicants' standard Multiplex PCR Master Mix containing "Q" solution were successful in simultaneously amplifying the 16 nucleic acid targets and rendering comparable amounts of amplicon. The Applicants' "Q solution" contains a polymeric volume exclusion agent as disclosed, exemplified and claimed in the application. It is these polymeric volume exclusion agents that are the "special multiplex PCR-enhancing synthetic factor[s]" that "eliminate the need for optimization – even when using equimolar primer concentrations." *See* Engel at p. 2, left col.

Applicants respectfully point out that the skilled artisan would have assumed that in order to obtain similar results using the prior art methods, they would have been required to perform the potentially endless trial and error experimentation – described herein and in the previous response filed October 22, 2009 – with no guaranty and no reasonable expectation of arriving at results similar to what is shown in the right two lanes of each panel above and in

Figures 1 and 2 of the current application. Surprisingly, however, the Applicants' methods are "fast and easy to perform."

Moreover, Applicants respectfully submit that the Office Action's citation of Groendahl et al., Journal of Clinical Microbiology, Jan. 1999, p. 1-7, ("Groendahl") further substantiates the unexpected nature of the results achieved using the claimed methods.

The Office Action indicates that Groendahl teaches a method of co-amplifying several different target nucleic acids. However, Groendahl fails to disclose the use of any polymeric volume exclusion agents.

Groendahl states that the object of their study was to "use an RT-PCR protocol that allows the simultaneous detection, within 1 day, of respiratory pathogens." At p. 1, right column. As a result, Groendahl developed and tested the single-tube m-RT-PCR-ELISA described in the paper. Groendahl state that, "[p]ublished multiplex PCR assays for the simultaneous detection of pathogens … and multiplex RT-PCR assays have included only two or three different organisms." Groendahl at p. 1, right col.

Groendahl demonstrates the development of a multiplex PCR assay capable of simultaneously amplifying 9 different target nucleic acids from a variety of microorganisms, warranted publication in a peer-reviewed journal *in its own right*. The person of skill in the art would have thought that the authors in Groendahl needed to carry out extensive optimization experiments in order to establish the described protocol. This is particularly true in view of Groendahl's statement that:

> for some organisms [i.e., target nucleic acids] the sensitivity was reduced by up to a factor of 10 in the m-RT-PCR approach. This is probably due to the accumulation of by-products through unspecific annealing during PCR if the annealing temperature is suboptimal for some templates.

Groendahl at p. 5, left col. It was well known that preferential amplification of certain targets in multiplex PCR experiments results in an imbalance of target amplicon yield formation. A person of skill in the art would, therefore, have believed that extensive and tedious optimization was required to find reaction conditions in which the accumulation of by-products from unspecific annealing was kept sufficiently low so as not to interfere with the other parallel amplifications. Indeed the current application states that:

> [s]trong differences in product yield are due to differences in hybridization [sic] of the primers to their respective target sequence and extension of such annealed primers. When such differences in product yield are observed, researchers typically need to adapt the concentration of primers to obtain comparable product yield. Establishment of the optimal molar ratio of primers is typically *very difficult* to perform. (emphasis added)

Application at paragraph [00023].

Applicants respectfully assert that Groendahl does not teach or disclose any generally applicable advances in the field of multiplex PCR. Specifically, there is nothing in Groendahl that would lead one of skill in the art to assume that establishing a protocol for the co-amplification of 9 target nucleic acids different from those disclosed therein, would require any less of the tedious and potentially open-ended optimization (e.g., described in the QIAGEN News cartoon above) associated with multiplex PCR at the time of filing of the current application.

Therefore, the skilled artisan would have been surprised by the results achieved by the claimed methods because they *require little or no optimization*. In particular, the claimed polymeric volume exclusion agents "eliminate the need for optimization – *even when using equimolar primer concentrations*." *See* Engel at p. 2, left col.

Clearly the claimed methods demonstrate a superior advantage that the person of ordinary skill in the relevant art would have found surprising or unexpected in 2002. *Soni,* 54 F.3d at 750. Accordingly, the Applicants respectfully assert that the subject Application provides unexpectedly superior results that are sufficient rebut any *prima facia* case of obviousness. *Id.*

Since market introduction of the Applicant's products and methods (e.g., QIAGEN® Multiplex PCR Kit, QuantiTect Multiplex PCR & RT-PCR Kits, QuantiFast Multiplex PCR & RT-PCR Kits, RotorGene Multiplex PCR & RT-PCR Kits, Type-IT product line, QuantiTect Virus PCR Kits, FastLane Cell Multiplex Kits, QIAgility Kits, QIAxcel Kits, HotStarTaq Master Mix Kit, etc.) covered by the claims at issue, multiplex PCR has been become routine. The assignee of the current application, Qiagen GmbH, is recognized as a worldwide leader in supplying multiplex PCR reagents. The QIAGEN®Multiplex PCR kits referenced in QIAGEN News and Engel have enjoyed enormous commercial success and have generated millions of

dollars annual revenue. A search of Google Scholar using the query "Qiagen Multiplex" returns 996 hits (Exhibit C).

Based on at least the arguments set forth above, Applicants respectfully request that the rejection of the claims under 35 U.S.C. § 103(a) be reconsidered and withdrawn.

II. The Rejection of Claims 12-15, Under 35 U.S.C. § 103(a) Should be Withdrawn

Claims 12-15 are rejected under 35 U.S.C. 103(a) as allegedly obvious over Backus in view of Bustin and Birch and further in view of Reed *et al.* U.S. Patent No. 5,459,038 (hereinafter "Reed") and Demke *et al.*, *Biotechniques 12*:333-334 (1992) (hereinafter "Demke"). Applicants respectfully traverse this rejection.

According to the Office Action Backus, as modified by Bustin and Birch, allegedly disclose the presently claimed invention as set forth above. However, the Office Action concedes that these references do not disclose the use of dextran as a volume exclusion agent. The Office Action attempts to cure this deficiency with the disclosures of Reed and Demke. The Office Action concludes that it would have been obvious to one of ordinary skill in the art to have extended the method of Backus to include dextran as discussed in Reed and Demke to achieve efficient amplification with higher sensitivity and specificity. Applicants respectfully disagree with these contentions and conclusions.

As set forth above and in previous argumentation, Applicants submit that the Office Action has not set forth a proper *prima facie* case of obviousness, as there would not have been a reasonable expectation of success of modifying the disclosure of Backus with that of Bustin and Birch. In addition, even if a *prima facie* case of obviousness was established, the unexpected results described herein clearly rebut this showing.

Applicants submit that these deficiencies are not cured by the disclosures of Reed and Demke, and hence, none of the cited references, alone or in combination, disclose this presently claimed invention. In view of the foregoing remarks, Applicants respectfully request reconsideration and withdrawal of this rejection.

III. The Rejection of Claim 25, Under 35 U.S.C. § 103(a) Should be Withdrawn

Claim 25 is rejected under 35 U.S.C. 103(a) as allegedly obvious over Backus in view of Bustin and Birch and further in view of Ivanov *et al.*, U.S. Patent No. 6,183,998 (hereinafter "Ivanov"). Applicants respectfully traverse this rejection.

According to the Office Action Backus, as modified by Bustin and Birch, allegedly disclose the presently claimed invention as set forth above. However, the Office Action concedes that these references do not disclose a chemically modified DNA polymerase. The Office Action attempts to cure this deficiency with the disclosure of Ivanov, arguing that Ivanov discloses reversible modification of DNA polymerases through reaction with an aldehyde. Applicants respectfully disagree with these contentions and conclusions.

As set forth above and previously argued, Applicants submit that the Office Action has not set forth a proper *prima facie* case of obviousness, as there would not have been a reasonable expectation of success of modifying the disclosure of Backus with that of Bustin and Birch. In addition, even if a *prima facie* case of obviousness was established, the unexpected results described herein clearly rebut this showing.

Applicants submit that these deficiencies are not cured by the disclosure of Ivanov, and hence, none of the cited references, alone or in combination, disclose this presently claimed invention. In view of the foregoing remarks, Applicants respectfully request reconsideration and withdrawal of this rejection.

IV. The Rejection of Claims 26-27, Under 35 U.S.C. § 103(a) Should be Withdrawn

Claims 26-27 are rejected under 35 U.S.C. 103(a) as allegedly obvious over Backus in view of Bustin and Birch and further in view of Mansfield *et al.*, *Molecular Cellular Probes* 9:145-156 (1995) (hereinafter "Mansfield"). Applicants respectfully traverse this rejection.

According to the Office Action Backus, as modified by Bustin and Birch, allegedly disclose the presently claimed invention as set forth above. However, the Office Action concedes that these references do not disclose that one of the primers is labeled with a specific binding moiety. The Office Action attempts to cure this deficiency with the disclosure of Mansfield, arguing that Mansfield discloses a variety of primer labeling techniques. Applicants respectfully disagree with these contentions and conclusions.

As set forth above and previously argued, Applicants submit that the Office Action has not set forth a proper *prima facie* case of obviousness, as there would not have been a reasonable expectation of success of modifying the disclosure of Backus with that of Bustin and Birch. In addition, even if a *prima facie* case of obviousness was established, the unexpected results described herein clearly rebut this showing.

Applicants submit that these deficiencies are not cured by the disclosure of Mansfield, and hence, none of the cited references, alone or in combination, disclose this presently claimed invention. In view of the foregoing remarks, Applicants respectfully request reconsideration and withdrawal of this rejection.

V. The Rejection of Claims 30-34, Under 35 U.S.C. § 103(a) Should be Withdrawn

Claims 30-34 are rejected under 35 U.S.C. 103(a) as allegedly obvious over Backus in view of Bustin and Birch and further in view of Groendahl *et al.*, *J. Clin. Micro.* *37*:1-7 (1999) (hereinafter "Groendahl"). Applicants respectfully traverse this rejection.

According to the Office Action Backus, as modified by Bustin and Birch, allegedly disclose the presently claimed invention as set forth above. However, the Office Action concedes that these references do not disclose the simultaneous amplification of six or eight targets. The Office Action attempts to cure this deficiency with the disclosure of Grondahl, arguing that Grondahl discloses coamplifying six or eight different target nucleic acids. Applicants respectfully disagree with these contentions and conclusions.

As set forth above and previously argued, Applicants submit that the Office Action has not set forth a proper *prima facie* case of obviousness, as there would not have been a reasonable expectation of success of modifying the disclosure of Backus with that of Bustin and Birch. In addition, even if a *prima facie* case of obviousness was established, the unexpected results described herein rebut this showing.

Applicants submit that these deficiencies are not cured by the disclosure of Grondahl, as Grondahl does not disclose that the maximum difference between the lowest and highest copy number is 10-fold, as required in the presently claimed invention. Hence, none of the cited references, alone or in combination, disclose this presently claimed invention. In view of the

foregoing remarks, Applicants respectfully request reconsideration and withdrawal of this rejection.

VI. Conclusion

Applicants believe that the claims are in condition for allowance and respectfully request allowance thereof. The Examiner is invited to telephone the undersigned if that would be helpful in resolving any issues.

It is not believed that extensions of time or fees for net addition of claims are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 50-5071.

Respectfully submitted,

Date: June 23, 2010

/Thomas Haag/

Thomas A. Haag, Ph.D.,Esq.
Registration No. 47,621
Attorney for Applicants

Fanelli Strain & Haag PLLC
Customer No. 0094136
1455 Pennsylvania Avenue, N.W.
Suite 400
Washington, D.C. 20004
Telephone: 202.621.1840
Facsimile: 202.621.1844